ORIGINAL ARTICLE

CONTENTS

Histological manifestations in the structures of the anterior abdominal wall after implantation of the acellular dermal matrix using the sublay method

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ABSTRACT

Aim: The objective of this study was to assess the level of vascularization, integration of the acellular dermal matrix, as well as the development of granulation tissue and collagen fibers following implantation on the anterior abdominal wall ("sub lay") in pigs over the course of 7, 14, and 21 days.

Materials and Methods: The experiment was conducted on six pigs of the Ukrainian White steppe. Under general anesthesia, an acellular perforated dermal matrix from pigs, produced by the «Institute of Biomedical Technologies», was implanted into the anterior abdominal wall, between the aponeurosis and muscles. The quality of engraftment, degree of angiogenesis, condition of granulation tissue, and graft structure were assessed. The postoperative period was uncomplicated, with each pig receiving one intramuscular dose of ceftriaxone (1.0). Material was collected from two pigs on the 7th day, two more on the 14th day, and the final two on the 21st day after implantation. Pathomorphological analysis was carried out in the laboratory of I. Horbachevsky Ternopil National Medical University. Sections of the implanted material, stained with hematoxylin-eosin, were evaluated for local inflammation reactivity, vascularization levels, and granulation tissue replacement.

Results: The research findings revealed that, after 7 days of implantation, the acellular skin matrix "sub lay" led to infiltration caused by acute inflammation, accompanied by the formation of granulation tissue, numerous microcirculatory vessels, and collagen fibers. By day 14, the signs of inflammation had reduced, and there was an increase in fibroblasts and blood vessels. On day 21, the implantation process intensified, marked by a rise in collagen fibers and vascularization, along with a decrease in macrophages and lymphocytes. No signs of infection were observed.

Conclusions: Acellular dermal matrix after «sub lay» implantation causes acute inflammation with slow vascularization in the early period with a moderate increase up to 14 and 21 days. The implant acts as a base to support the migration of natural cells and subsequent replacement by granulation tissue, as a result of strengthening this area. No signs of infection were detected, which gives hope for the use of implants in previously infected environments after complications due to the implantation of meshes made of artificial materials before.

KEY WORDS: Acellular dermal matrix, pigs, "sub lay" implantation, microscopy

Wiad Lek. 2025;78(4):821-827. doi: 10.36740/WLek/203892 DOI

INTRODUCTION

Surgical reconstruction of the abdominal wall is one of the most common needs in hernia surgery due to both tissue deficiency and high suture line tension, which leads to postoperative hernias [1]. Incisional hernias are a common complication of laparotomy incisions and a major healthcare burden. The management of incisional hernias are challenging and complex. This activity reviews preoperative optimization, techniques for repair, mesh use, and complications of incisional hernia repair and highlights some strategies to reduce the rate of incisional hernia.

Treatment strategy for hernia involves surgical repair or conservative treatment. The decision to choose between the two options depends on a few factors like symptoms, the size of a hernia, complications and patients' preference. Open, laparoscopic technique has been commonly used to repair incisional hernias. Mesh provides the strength for the repair and scaffold for the healing tissue. Meshes are characterized as permanent vs. absorbable, and synthetic vs. biologic.

The use of polypropylene mesh for the surgical treatment of hernia defects of the abdominal wall helps to reduce recurrence [2, 3]. However, this is accompanied by several complications, such as increased postoperative pain, abdominal adhesions, and areas of fibrosis, and an increased risk of infection due to prosthesis placement [1, 4].

Since the beginning of the last century, the use of prostheses has become a constant in the surgery of abdominal wall hernias especially inguinal hernias [4]. The study and search for alternative materials, such as

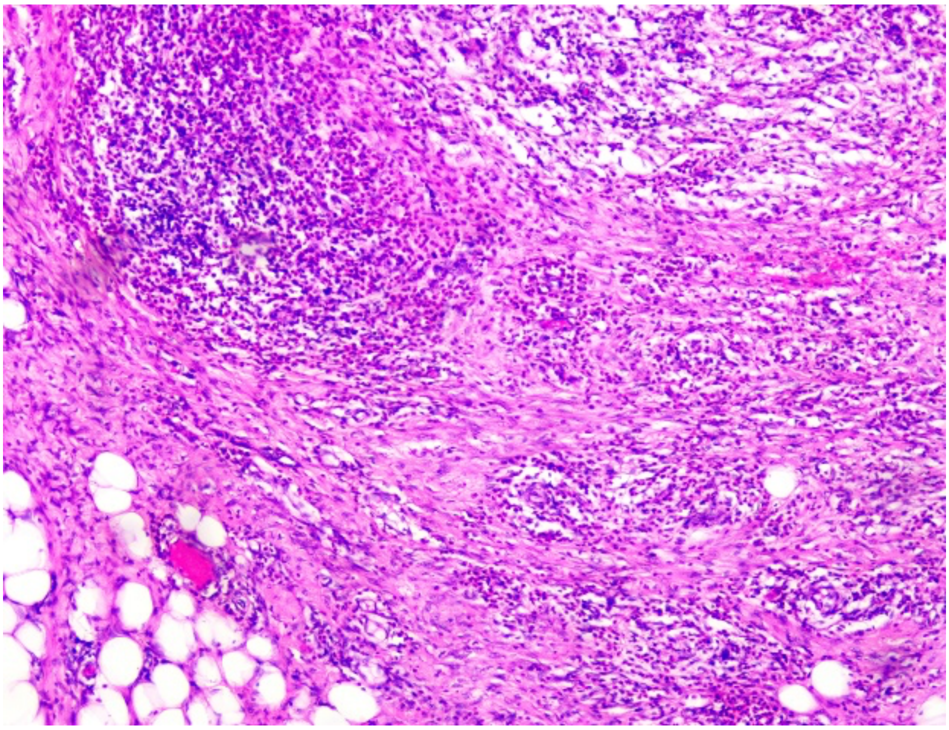

Fig. 1. The area of aponeurosis treated with a porcine skin xenograft using the sublay method after 7 days of experiment. Inflammatory infiltration of granulation tissue with the formation of leukocyte infiltration foci. Hematoxylin and eosin, x 100.

bovine pericardium, dermal matrix from pig skin, human skin, have proven to be one of the options for hernia repair and treatment, leading to better results[5-7].

Complete evaluation of incisional hernias includes confirming the diagnosis, sizing the defect, identifying the herniated content, and assessing the abdominal cavity to plan the surgical treatment in complex hernias. CT scan imaging is useful for obtaining these details [8, 9].

However, there are insufficient data on the reaction of local tissues to the implant, the level of vascularization, integration and the formation of collagen fibers after implantation at different levels over some time [10, 11].

AIM

The objective of this study was to assess the level of vascularization, integration of the acellular dermal matrix, as well as the development of granulation tissue and collagen fibers following implantation on the anterior abdominal wall ("sub lay") in pigs over the course of 7, 14, and 21 days.

MATERIALS AND METHODS

The experiment was performed on 6 pigs of the Ukrainian White steppe. Under general anesthesia, acellular perforated dermal matrix of a pig produced by "Institute of Biomedical Technologies" (Chief Director Prof. Bigunyak V.V.) was implanted on the anterior abdominal wall in the space between the aponeurosis and muscles ("sub lay") The quality of engraftment, degree of angiogenesis, condition of granulation tissue and graft structure were estimated.

The postoperative period was uncomplicated, every pig received 1 dose of ceftriaxone 1.0 intramuscularly. From 2 pigs the material was collected on the 7th day, another 2 on the 14th day, and in the last 2 on the 21st day after implantation.

Pathomorphological research were done in the laboratory of I. Horbachevsky Ternopil National Medical University. Sections of the implanted material were stained with hematoxylin-eosin were evaluated concerning the reactivity of local inflammation, the level of vascularization, and replacement by granulation tissue.

RESULTS

HISTOLOGICAL CHANGES OBSERVED IN THE STRUCTURES OF THE ANTERIOR ABDOMINAL WALL FOLLOWING ACELLULAR DERMAL MATRIX IMPLANTATION USING THE «SUBLAY» TECHNIQUE OVER VARIOUS TIME PERIODS (7, 14, 21 DAYS)

The implantation of the acellular dermal matrix (lyophilized pig skin) was performed in the anterior abdominal wall, specifically in the space between the aponeurosis and muscles (sublay technique).

Seven days after the implantation, well-formed granulation tissue was noted, accompanied by intense inflammatory infiltration and focal areas of leukocyte infiltration (Fig. 1). A significant presence of microcir-

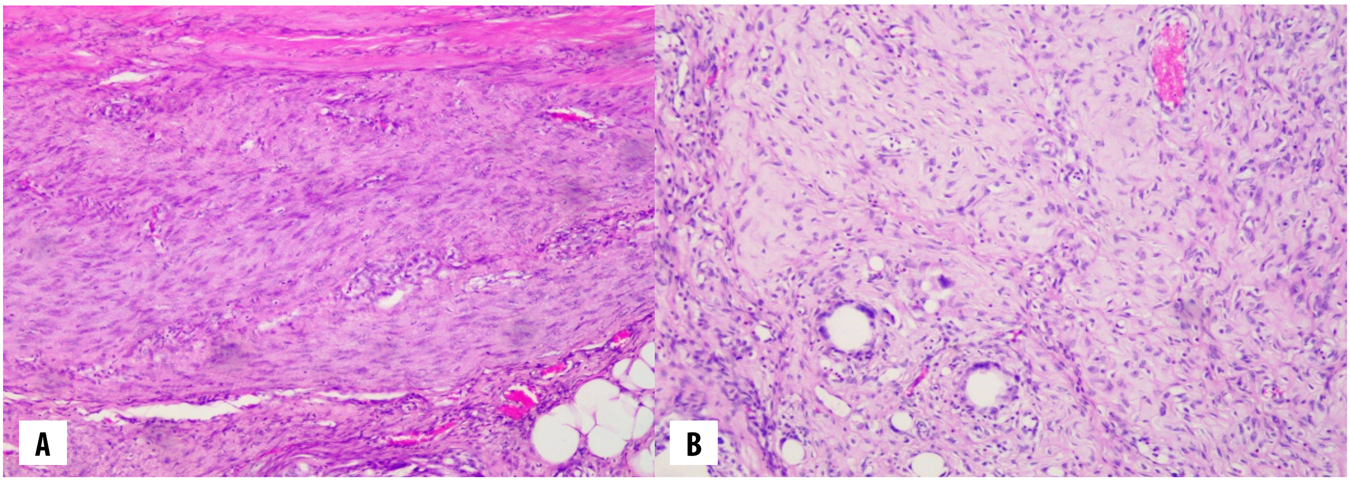

Fig. 2. The area of aponeurosis treated with a porcine skin xenograft using the sublay method after 14 days of experiment. Granulation tissue with mild inflammatory infiltration and an increased number of fibroblasts (A), the granulation tissue is formed with well-defined blood vessels (B).

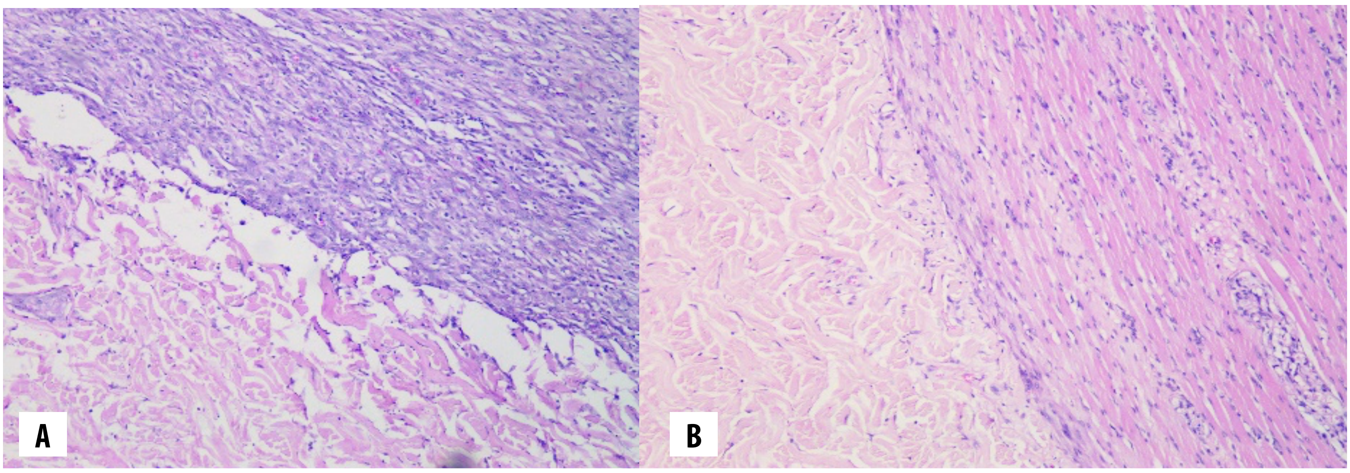

Fig. 3. The area of aponeurosis with the use of porcine skin xenograft according to the method (sublay) after 21 days of the experiment. Fibrinoid edema of collagen fibers with foci of necrosis (A), Collagen fibers are moderately organized. Moderate inflammatory infiltration of the stroma (B). Hematoxylin and eosin, x 100.

culatory vessels and collagen fibers was observed. The inflammatory response was particularly pronounced, with some areas showing necrosis at the graft contact points. The number of macrophages and fibroblasts remained low, while lymphocytic activity was moderate.

There was minimal inflammatory infiltration with clusters of polymorphonuclear leukocytes in the detected adipose tissue.

The graft`s structure was partially altered in the regions of contact with the tissue, as indicated by the presence of mucoid edema in the fibers. In other areas, the structure consists of well-preserved fibers arranged in an orderly manner, with minimal focal lymphocyte infiltration.

Histologic examination of the tissue 14 days after xenograft implantation revealed the formation of granulation tissue, including numerous microcirculatory vessels and a moderate amount of collagen fibers. The signs of inflammatory infiltration diminished, with lymphocytes and histiocytes being the predominant cells, while the number of the leucocytes significantly decreased (Fig. 2). Blood filling in the vessels was uneven, with some lumens still dilated, but no perivascular edema was noted. The overall density of microcirculatory vessels was was considerable.

Perivascular edema and moderate lymphohistiocytic infiltration, including occasional macrophages, were observed in the areas where the graft made contact. The inflammatory infiltration extended slightly into the surrounding tissue. Within the graft structure, individual capillaries surrounded by lymphocytes and histiocytes were visible.

Histologic examination of the aponeurosis area 21 days after acellular dermal matrix implantation showed a moderate reduction in inflammatory infiltration within the intervention zone. The granulation area had significantly redused, primarily due to decreasing inflammatory infiltration, perivascular edema and capillary blood filling. The area of collagen fibers slightly increased, with the fibers arranged in a more orderly fashion (Fig. 3).

Macrophages, lymphocytes, histiocytes, and fibroblasts were observed among the fibers, with a reduction in the number of fibroblasts. Within the graft structure, the density of microcirculatory vessels sprouting from the granulation tissue moderately increased. In some areas, fibrinoid edema and focal necrosis of the collagen stroma were evident, while in other areas, focal inflammatory infiltration along the collagen stroma was visible. So, the implant acts as a base to support the migration of connective tissue cells and subsequent replacement by granulation tissue, as a result of strengthening this area.

DISSCUSION

Critical review of the literature revealed a large variety in mesh models; many different models, animal species, meshes, and parameters were assessed in the last decade leading to studies that were difficult to compare among each other. Identical models including all parameters were not found to be implemented by different centers, in other words all centers apparently use their own specific models. Due to the growing variety in existing and new concepts of meshes, preclinical animal research is necessary to assess biocompatibility and effectiveness of new meshes before implementing them in clinical practice [12-14]. Furthermore, many of the important mesh characteristics are derived from and can only be properly researched using animal models [15, 16]. However, for experimental research to have proper impact, research published by different research groups needs to be comparable and reproducible [17, 18].

In laparoscopic incisional hernia repair, direct contact between the prosthesis and abdominal viscera is inevitable and may lead to adhesions [19-21]. Despite the large variety of mesh prosthesis, little is known about their in vivo behavior. Biological meshes are considered to have many advantages, but due to their price they're rarely used. A rat model was used to assess biological and conventional synthetic meshes on their in vivo characteristics. Based on incorporation, adhesion surface, adhesion strength, mesh shrinkage, and the histologic parameters scaffold degradation, cellular infiltration, neovascularization, and extracellular matrix deposition, Strattice™ performed best in this experiment in a physiologic, non-contaminated rat model with intraperitoneal mesh placement.

Biological grafts, introduced as alternatives to synthetic commercial meshes for hernia repair, offer the potential for fewer post-operative complications compared to synthetic options [22-24]. These grafts are made through tissue decellularization and can be derived from human (allogenic) [25, 26] or animal (xenogenic) sources, including dermis from both species [27-29], porcine small intestinal submucosa [30-32], and bovine pericardium [33-35], or other organs. Decellularization methods – such as physical, chemical, and enzymatic processes – help prevent infections and foreign body reactions. Some commercially available biological tissue grafts for abdominal wall hernia repair include Permacol™, Surgisis®, SurgiMend™, XenMatrix™, Flex-HD™, Veritas®, AlloMax™, Periguard®, and Alloderm®.

There are several basic methods for placing implants in reconstructive surgery of abdominal wall defects: Onlay (on the aponeurosis), Sublay (under the aponeurosis), PPT (preperitoneally), and several modifications of these methods (e.g., Inlay). All methods are highly effective and are commonly used in the practical work of surgeons. Each method has its own advantages and disadvantages [3, 15].

A significant number of complications are caused by the type of material used for the prosthesis [13]. The main aim of our research is to investigate the integration and engraftment of biological implants, specifically the acellular dermal matrix of pigs, when implanted at different levels of the abdominal wall. Here we showed the pathomorphological results of the implantation sublay of porcine dermal matrix.

Extracellular collagen matrices in biological grafts promote quick tissue ingrowth and neovascularization, encouraging strong, durable integration with the patient's own tissues. As the grafts integrate, they degrade gradually and remodel into newly regenerated tissue that supports the abdominal cavity. A study by Ghetti et al. assessed the post-operative morphological changes in hernia patients one year after receiving human decellularized matrices. Their findings revealed regenerative cellular recruitment, neovascularization, reduced inflammation, and a well-organized collagen matrix at the implant site [36]. In another study, Hoganson and colleagues developed an acellular porcine dermis matrix that successfully preserved ECM components such as proteins, glycosaminoglycans, and cytokines (e.g., VEGF, TGF-β), all of which aid in graft integration and wound healing [37]. Co-cultured fibroblasts showed typical spindle-like shapes and strong viability after one and two weeks. The presence of bioactive components and appropriate mechanical strength suggests that decellularized dermis materials have significant clinical potential. Additionally, biological meshes have been found to resist infection when implanted, with no need for removal even if infection occurs [37, 38]. For this reason, biological meshes are particularly beneficial in addressing body trunk defects and complex cases where infection risks are high.

Faster vascularization and cell migration, with subsequent formation of granulation tissue, were observed after the Sublay method of surgery compared to the Onlay method. Moderate inflammation and the absence of infection following the Sublay technique are significant advantages for promoting high-quality healing.

Therefore, studies on the integration of biological implants after their implantation at different levels of the abdominal wall are promising for future research [38, 39].

CONCLUSIONS

Acellular dermal matrix after «sub lay» implantation causes acute inflammation with slow vascularization in the early period with a moderate increase up to 14 and 21 days. The implant acts as a base to support the migration of cells and subsequent replacement by granulation tissue, as a result of strengthening this area. No signs of infection were detected, which gives hope for the use of implants in previously infected environments after complications due to the implantation of meshes made of artificial materials before.

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The work was carried out according to the research work of I. Horbachevsky Ternopil National Medical University "Information technologies DATA SCIENCE and BIG DATA in the cyber-physical systems of medical biological processes" (state registration number 0122U000030).

CONFLICT OF INTEREST
The Authors declare no conflict of interest

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RECEIVED: 21.10.2024
ACCEPTED: 24.03.2025